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On April 21, 2006

TOWNSEND and TOWNSEND and CREW LLP

By: Patricia Anders

PATENT  
Attorney Docket No.: 022101-001900US  
Client Ref. No.: 19094-US

**IN THE UNITED STATES PATENT AND TRADEMARK OFFICE**

In re application of:

Gregor SAGNER et al.

Application No.: 09/823,712

Filed: March 30, 2001

For: METHOD FOR DETERMINING  
THE EFFICIENCY OF NUCLEIC ACID  
AMPLIFICATIONS

Customer No.: 41504

Confirmation No. 7485

Examiner: Suryaprabha Chunduru

Technology Center/Art Unit: 1637

DECLARATION OF CARL WITTWER,  
PH.D., M.D. UNDER 37 C.F.R. §1.132

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Sir:

I, Carl Wittwer, Ph.D., M.D., being duly warned that willful false statements and the like are punishable by fine or imprisonment or both (18 U.S.C. § 1001), and may jeopardize the validity of the patent application or any patent issuing thereon, state and declare as follows:

1. All statements herein made of my own knowledge are true, and statements made on information or belief are believed to be true and correct.

2. I received my Ph.D. in the field of Biochemistry from Utah State University in 1982 and an M.D. at the University of Michigan in 1984. I am currently a professor in the Department of Pathology at the University of Utah School of Medicine. I have been in this position since 1988. I am also a cofounder of Idaho Technology, Inc., a company supplying PCR products. A copy of my curriculum vitae is attached hereto as Exhibit A.

3. I have reviewed U.S. Patent Application No. 09/823,712 (the '712 application) as well as the office actions dated July 13, 2005 and December 23, 2005 and the Applicant's response dated October 4, 2005. I understand that the claims, as amended in the concurrently filed amendment, include a step involving determination of a non-linear continuously differentiable function of a logarithm of the initial copy number of target nucleic acid in a dilution series used for amplification as a function of the cycle number at which the signal threshold value is exceeded.

4. I understand that the Examiner has rejected the pending claims as obvious over Lowe *et al.*, WO 99/54510 in view of Wittwer *et al.*, U.S. Patent No. 6,174,670 ("the '670 patent"). I am an inventor of the '670 patent. According to the Examiner, Lowe *et al.* describes a number of the steps of the claims of the '712 application, but does not describe determining a non-linear continuously differentiable function of a logarithm of the copy number of target nucleic acid used for amplification as a function of the cycle number at which the signal threshold value is exceeded. However, the Examiner has argued that the '670 patent described "DNA monitoring at each PCR cycle by measuring melting curves and calculating copy number at each cycle utilizing a DNA-binding dye (SYBR Green I)," and concludes that the '670 patent teaches a non-linear function that would be obvious to combine with the Lowe reference

to achieve the presently claimed invention. *See*, December 23, 2006 office action, page 4, second and third paragraphs.

5. I disagree with the Examiner because the '670 patent does not describe generating a non-linear function of the logarithm of initial copy number and the cycle threshold (the cycle number at which the signal threshold value is exceeded). The Examiner correctly states that the '670 patent describes a method of DNA monitoring at each PCR cycle by measuring melting curves and calculating copy number at each cycle. However, the claimed invention involves determining a non-linear function of the logarithm of the initial concentration of nucleic acid in multiple dilutions and the cycle threshold. Monitoring amplification at each cycle, as described in the cited sections of the '670 patent, does not render it obvious to determine a non-linear function of the logarithm of initial copy number and cycle threshold.

6. The sections of the '670 patent cited by the Examiner (col. 3, lines 30-61; col. 4, line 45-63; col. 7, line 14-31; Figs. 22-23; and col. 17, lines 34-39) describe monitoring of amplification in real time (i.e., at every amplification cycle), but these sections do not teach or suggest the claimed non-linear relationship of the logarithm of initial copy number and cycle threshold. For example, the Examiner has cited col. 4, lines 45-63 of the '670 patent, which refers to a "3-dimensional spiral," for a teaching of "nonlinear functionality." *See*, December 23, 2005 Office Action, page 4, third paragraph. While the '670 patent does indeed refer to a "3-dimensional spiral," the spiral has nothing to do with the invention as currently claimed in the '712 application. Rather than teaching anything about the relation of the logarithm of initial copy number and cycle threshold, the "3-dimensional spiral" refers to measurement of temperature, time and fluorescence during each cycle of an amplification. *See*, the '670 patent, col. 4, lines 52-63. Measurement of temperature, time and fluorescence *within* an amplification does not suggest the relation of the logarithm of initial copy number and cycle threshold

because these measurements do not involve analysis of dilutions of a target nucleic acid. Indeed, the use of the term "3-dimensional" does not relate at all to a mathematical function as recited in the claims of the '712 application. Therefore the Examiner is not correct in stating that the "3-dimensional spiral" discussed in the '670 patent has anything to do with the non-linear functions recited in the claims of the '712 application.

7. Figures 22 and 23 of the '670 patent, also cited in the Examiner's rejection, do not teach or suggest determining a non-linear function of the logarithm of initial copy number and cycle threshold. Figure 22 displays real time fluorescence of an amplification of one sample. Fluorescence information from one amplification does not provide information regarding the cycle threshold values from *different* dilutions that one of skill in the art would use to determine a function between the logarithm of copy number and cycle threshold.

8. Figure 23 displays real time fluorescence in relation to cycle number for different dilutions of a target nucleic acid. However, determining a non-linear function of the logarithm of initial copy number and cycle threshold is not suggested in Figure 23. Moreover, it was commonly assumed before the filing of the '712 application that a *linear* function of the logarithm of initial copy number to cycle threshold should be determined. This is illustrated, for example, in the Lowe *et al.* reference (page 5, lines 7-8 and Figure 1B), each of which use *linear* regression to determine a *linear* function between the logarithm of initial copy number and cycle threshold, and in Figures 24, 26, and 28 of the '670 patent, which suggests good fit to a linear function. Nothing in the art the Examiner has cited contradicts this common assumption, i.e., that one of skill in the art should generate a *linear* function of the logarithm of initial copy number and cycle threshold.

9. Column 40, lines 14-24 of the '670 patent notes a decrease in reaction efficiency when initial copy number is 100 copies or below. However, this result was interpreted as a result of primer dimers and nonspecific amplification (col. 40, lines 24), and the data from samples with low initial copy number were normalized using a value generated from melting peak integration (col. 40, lines 40-47). This is illustrated, for example, in Figure 42A, which illustrates the original data, and Figure 42D, which shows the normalized data. The normalized data presented in Figure 42D from the various dilutions are shown as amplification curves that have approximately equal spacing, even at low dilutions, in contrast to the original data displayed in Figure 42A. While not graphically represented as a logarithm of the copy number of target nucleic acid as a function of the cycle number at which the signal threshold is exceeded, this normalization process as described in col. 40 and Figure 42, would tend to linearize such a function.

10. In view of the forgoing, it is my scientific opinion that the claimed invention in the '712 application is not obvious. The combination of the Lowe *et al.* reference and the '670 patent does not teach or suggest a step of determining a non-linear continuously differentiable function of logarithm of initial copy number as a function of cycle threshold.

Date: April 6, 2006

By: Carl Wittwer

Carl Wittwer, Ph.D., M.D.

## Curriculum Vitae

### I. PERSONAL DATA

Carl Thomas Wittwer  
Born March 8, 1955, Lansing, MI, USA  
US Citizenship  
Ethnicity: White  
SSN: 366-64-4751

### II. EDUCATION/LICENSURE

Middlebury College, Middlebury, Vermont College Scholar	1973-1975
Utah State University, Logan, Utah BS Chemistry, PhD Biochemistry	1975-1978 1980-1982
University of Michigan, Ann Arbor, Michigan MD	1978-1980 1982-1984
State of Utah Medical License	1984
University of Utah, Salt Lake City, UT, Residency	1984-1988
Board Certified in Anatomic and Clinical Pathology	1988
New York State Certification for genetic testing, molecular oncology, immunohematology, and cellular immunology	1992-

### III. PROFESSIONAL EXPERIENCE

#### A. Full Time Positions:

Professor Department of Pathology University of Utah School of Medicine	2000-
Associate Professor Department of Pathology University of Utah School of Medicine	1994-2000
Assistant Professor Department of Pathology University of Utah School of Medicine	1988-1994

#### B. Part Time Positions:

Chief Science Officer/Vice President for Research Idaho Technology Salt Lake City, UT	1990-
Medical Director Myriad Diagnostics, Salt Lake City, UT	1993-1996

Adjunct Assistant Professor Department of Nutrition and Food Science Utah State University	1985-2000
NIH Study Section, Biological and Physiological Sciences Special Emphasis Panel, SSS-Y	1997-
NCI Study Section and Site Visit Reviewer	1998-
Molecular consultant (Various companies)	1999-

#### C. Editorial Experience:

1. Clinical Chemistry, Board of Editors, 2000-
2. Clinical Chemistry, Associate Editor, 2002-
3. Rapid Cycle Real-Time PCR -- Methods and Applications. S Meuer, C Wittwer, K Nakaguwara, eds., Springer, Berlin, 2001.
4. Molecular Testing in Laboratory Medicine: Selections from Clinical Chemistry, 1998-2001, with Annotations and Updates. DE Bruns, YMD Lo, and CT Wittwer, eds., AACC press, Washington, DC, 2002.
5. Rapid Cycle Real-Time PCR – Methods and Applications: Microbiology and Food Analysis. U. Reischl, C Wittwer, and F. Cockerill, eds., Springer, Berlin, 2002.
6. Rapid Cycle Real-Time PCR – Methods and Applications: Genetics and Oncology. Dietmaier, C. Wittwer, and Sivasubramanian, eds., Springer, Berlin, 2002.
7. Rapid Cycle Real-Time PCR – Quantification. C. Wittwer, M. Hahn, and K Kaul eds., Springer, Berlin, 2004.
4. Research article referee for the following journals:  
Am. J. Path.  
Analytical Biochemistry  
Anal. Chem.  
BioTechniques  
Biochim. Biophys. ACTA  
Clinical Chemistry  
Cytometry  
Human Mutation  
J. Mol.Diag.  
Nature Med.  
Nucl. Acids Res,  
Proc. Natl. Acad. Sci.

#### D. Research Awards:

Instrumentation for quantitative rapid cycle PCR, Technology Innovation Grant.  
University of Utah Research Foundation., Principal Investigator, 7/94-6/96, \$90,000.

Continuous monitoring of rapid cycle PCR. NIH STTR Phase I and Phase II Grants,  
Principal Investigator, 9/94-9/98, \$600,000.

Temperature cycling by adiabatic compression. Biomedical Engineering Grant. Whitaker Foundation, Principal Investigator, 12/95-11/98, \$210,000.

Fluorescent PCR techniques. Idaho Technology, Principal Investigator, 7/97-12/02, \$950,000.

Homogeneous multiplex PCR by fluorescence and T<sub>m</sub>. NIH STTR Phase I and II Grant, Principal Investigator, 4/1/99-2/03, \$620,000.

Single-Labeled Probes for Real-Time PCR. Technology Commercialization Project. University of Utah Research Foundation, Principal Investigator, 7/02-6/03, \$35,000.

Fluorescent PCR techniques. Idaho Technology, Principal Investigator, 1/03-12/07, \$1,650,000.

Center for Homogeneous DNA Analysis. State of Utah Centers of Excellence Grant, Principal Investigator, 7/03 – 6/08, \$150,000/year pending approval each year.

SNP analysis without probes. Technology Commercialization Project. University of Utah Research Foundation, Principal Investigator, 7/03-6/05, \$70,000.

Homogeneous mutation scanning. NIH Fast Track STTR, Principal Investigator, 8/04-1/07, \$850,000.

A system for rapid PCR, mutation scanning and genotyping. NIH Fast Track STTR, Principal Investigator, 3/05-9/07, \$850,000.

#### E. Patents and Copyrights:

DNALYSIS - DNA content and cell cycle analysis software, copyright 1989, University of Utah.

US Patent 5,455,175. Automated Rapid Temperature Cycling Device, University of Utah, 1995.

US Patent 5,935,522. On-line DNA analysis system with rapid thermal cycling, U. of Utah, 1999.

US patent 6,140,054. Multiplex genotyping using fluorescent hybridization probes, Univ. of Utah, 2000.

US patent 6,174,670. Monitoring amplification of DNA during PCR, U. Utah, 2001.

US patent 6,197,520. Solution-based color compensation adjusted for temperature and electronic gains, Univ. of Utah, 2001.

US patent 6,232,079. PCR method for nucleic acid quantification utilizing second or third order rate constants, U. Utah, 2001.

US patent 6,245,514. Fluorescent donor-acceptor pair with low spectral overlap, U. Utah, 2001.

US patent 6,303,305. Method of quantification of an analyte, U. Utah, 2001.

U.S. Patent 6,387,621. Automated analysis of real-time nucleic acid amplification, Univ. of Utah, 2002.



US patent 6,472,156. Homogeneous multiplex hybridization analysis by color and T<sub>m</sub>, Univ. of Utah, 2002.

US patent 6,503,720. Method for quantification of an analyte, Univ. Utah, 2003.

US patent 6,569,627. Monitoring hybridization during PCR using SYBR.<sup>TM</sup>. Green I, Univ. of Utah, 2003.

US patent 6,635,427. Single-labeled oligonucleotide probes for homogeneous nucleic acid sequence analysis, Univ of Utah, 2003.

US patent 6,730,501. Multi-test analysis of real-time nucleic acid amplification, Univ. Utah, 2004.

US patent 6,753,141. Simultaneous screening and identification of sequence alterations from amplified target, U. Utah, 2004.

US patent 6,787,338. Method for rapid thermal cycling of biological samples. U. Utah, 2004.

NZ patent 333136. Monitoring hybridization during PCR. U. Utah. 2000.

NZ patent 333137. System and method for carrying out and monitoring biological processes. U. Utah, 2000.

NZ patent 502323. Monitoring hybridization during PCR. U. Utah, 2002.

Australian patent 726501. Monitoring hybridization during PCR. U. Utah., 2001.

Australian patent 727296. systems and methods for monitoring for DNA amplification by fluorescence, U. Utah, 2001.

Australian patent 729644. System and method for monitoring PCR processes, 2001.

Japanese Patent No. 3670967. Multiplex Genotyping Using Fluorescent Hybridization Probes.

Published US patent application 20020151039 DNA amplification using electrolyte conductance heating and temperature monitoring, Univ. Utah.

Published US patent application. 20030165867 Multi-test analysis of real-time nucleic acid amplification, Univ. Utah.

Published US patent application. 20030224434 Genotyping by amplicon melting curve analysis, Univ. Utah.

Published US patent application. 20040002098 Monitoring amplification with fret probes, Univ. Utah.

Published US patent application. 20040033518 Characterization of single stranded nucleic acids by melting analysis of secondary structure using double strand-specific nucleic acid dye, Univ. Utah.

Published PCT patent application WO 2004/038038 A2. Amplicon melting analysis with saturation dyes, Univ. of Utah and Idaho Technology.

Published US patent application 20040265892 Method for rapid cycling of biological samples., Univ. Utah.

Published US patent application 20050034493 Method for rapid cycling of biological samples., Univ. Utah.

#### IV. HONORS AND AWARDS

Greenwood Memorial Scholarship in Biochemistry	1980
Cleveland Clinic Award for Medical Research	1984
FDA Physician Sponsor (Investigational New Drug Trials) for carnitine (IND #25,201) and pantethine (IND #25,416).	1986-2000
Young Investigators Award, ACLPS	1987
International presentations:	
Korea: Flow cytometry	1987-1989
Korea, Taiwan: Rapid PCR techniques	1992, 1994
Germany: Fluorescent PCR Techniques	1997-
Norway: Fluorescent PCR Techniques	1999
Outstanding Teaching Award, Clinical Pathology, Univ. of Utah	1994, 1996
Franklin Jefferson Award for Science, Technology, and Innovation (NIH STTR), The Small Business High Technology Institute.	1999
Scott and Dorothy Watkins Endowed Chair in Pathology Honoring Ernst Eichwald	1999-2002
Gordon Jensen Pathfinder Award Small Business/Inventors Conference, Salt Lake City, UT	2002
Governor's Medal for Science and Technology State of Utah	2003
Stoel Rives Innovation Award, First Place (BioTechnology) High Resolution Mutation Scanning	2004
AACC award for Outstanding Contributions to Clinical Chemistry in a Selected Area of Research	2004
Outstanding Speaker Award, AACC	2003, 2004
Innovation Award, Technology Transfer University of Utah	2004
Technical Advancement Award IQLM (International Quality in Laboratory Management) CDC/Atlanta	2005
IFCC-Abbott Award for Significant	2005

Contributions to Molecular Diagnostics  
IFCC/AACC

V. ADMINISTRATIVE EXPERIENCE

Medical Director Flow Cytometry Associated and Regional University Pathologists	1988-
Associate Director HLA Laboratory University of Utah	1988-1992
Technical Vice President of New Technology Associated Regional and University Pathologists Salt Lake City, UT 84108	1990-
Medical Director Molecular Diagnostics Associated and Regional University Pathologists	1992-1996
Medical Director Advanced Technology Group Associated and Regional University Pathologists	2001-

VIII. MEMBERSHIP IN PROFESSIONAL SOCIETIES

Society for Analytical Cytology  
Association for Molecular Pathology  
Academy of Clinical Laboratory Physicians and Scientists

IX. TEACHING RESPONSIBILITIES/ASSIGNMENTS

Pathology resident rotation in Flow Cytometry/ Molecular Pathology, 4-5 residents/year	1988-1999
Mentor to Resident and Fellow Research Projects with seven abstracts receiving Young Investigator Awards	1988-
Medical and graduate student guest lectures on flow cytometry, HLA typing, and DNA diagnostics.	1988-
Mentor for visiting fellows from Korea and Singapore	1994-
Major professor for students in Nutrition, Medical Laboratory Science and BioMedical Engineering.	
Panthenate-p-nitroanilide as a substrate for panthetheinase assay, Robert T. Davidson, Nutrition, Utah State University	1994

Rapid Cycle PCR for diagnosis of fragile X Syndrome, H. S. Lee, Med. Lab Sci, U of U.	1995
Continuous monitoring of the PCR for hepatitis B Virus: detection and quantification., Doug Searles, Med. Lab Sci., U of U.	1997
Fuzzy cluster image analysis of bone marrow morphology, R. K. Wang, Med. Lab Sci, U of U.	1998
Low temperature capillary fluorimeter for conformational analysis of nucleic acids, Wade Dummer, BioMedical Engineering, U of U	2000
Quenching of fluorescently-labeled probes upon hybridization Cameron Gundryt, Med Lab Sce, U of U	2003
ApoE genotyping by multiplex unlabeled probe melting analysis Matt Poulson, Med Lab Sci., U of U	2005
Genotyping four thrombophilia SNPs by multiplex small amplicon Melting without probes. Mike Seipp, Med Lab Sci, U of U	2006

#### PEER REVIEWED PUBLICATIONS:

1. Wyse BW, **CT Wittwer**, RG Hansen. Radioimmunoassay for pantothenic acid in blood and other tissues. Clin. Chem. 25:108-111, 1979.
2. **Wittwer CT**, BW Wyse, RG Hansen. Assay of the enzymatic hydrolysis of pantetheine. Anal. Biochem. 122:213-222, 1982.
3. **Wittwer CT**, Burkhard, K Ririe, R Rasmussen, J Brown, BW Wyse, RG Hansen. Purification and properties of a pantetheine-hydrolyzing enzyme from pig kidney. J. Biol. Chem. 258:9733-9738, 1983.
4. **Wittwer CT**, WA Gaul, JD Butler, M Zatz, JG Thoene. Metabolism of pantethine in cytinosis. J. Clin. Invest. 76:1665-1672, 1985.
5. Wyse BW, RG Hansen, CT Windham, **CT Wittwer**. The status of human nutrition and agricultural productivity. J. Home Econ. 78:19-24, 1986.
6. Gleeson JM, **CT Wittwer**, CA Schipke, DE Wilson. Effect of carnitine and pantethine on the metabolic abnormalities of acquired total lipodystrophy. Curr. Ther. Res. 41:83-88, 1987.
7. **Wittwer CT**, AM Smith, KO Ash, CW DeWitt. False-positive antibody tests for human immunodeficiency virus in transplant patients with antilymphocyte antibodies. Transplantation 44:843-844, 1987.
8. **Wittwer CT**, CP Graves, MA Peterson, E Jorgensen, JG Thoene, BW Wyse, CT Windham, RG Hansen. Pantetheine lipomodulation: evidence for cysteamine mediation in vitro and in vivo. Atherosclerosis 68:41-49, 1987.
9. Rabkin MS, CR Kjeldsberg, EG Hammond, **CT Wittwer**, B Nathwani. Clinical, ultrastructural, immunohistochemical, and DNA content analysis of lymphomas having features of interdigitating reticulum cells. Cancer 61:1594-1601, 1988.

10. Rabkin MS, **CT Wittwer**, CR Kjeldsberg, MW Piepkorn. Flow cytometric DNA-content analysis of histiocytosis X. *Am. J. Path.* 131:283-289, 1988.
11. **Wittwer CT**, MR Bristow, EM Gilbert, DG Renlund, JB O'Connell, CW DeWitt. OKT3 therapy as a cause of high panel reactive antibodies in serum using standard microcytotoxicity techniques. *Transplantation* 45:832-834, 1988.
12. Pearson SD, **CT Wittwer**, KO Ash. Evaluation of three commercial kits for the confirmation of antibodies to human immunodeficiency virus (HIV-1). *Clin. Chem.* 34:1930, 1988.
13. **Wittwer CT**, C Schweitzer, J Pearson, WO Song, CT Windham, BW Wyse, RG Hansen. Enzymes for liberation of pantothenic acid in blood: use of plasma pantothenase. *Am. J. Clin. Nutr.* 50:1072-1078, 1989.
14. **Wittwer CT**, GC Fillmore, DR Hillyard. Automated polymerase chain reaction in capillary tubes with hot air. *Nucl. Acids Res.* 17:4353-4357, 1989.
15. **Wittwer CT**, WA Knape, MR Bristow, EM Gilbert, DG Renlund, JB O'Connell, CW DeWitt. The quantitative flow cytometric plasma OKT3 assay - its potential application in cardiac transplantation. *Transplantation* 48:533-535, 1989.
16. Song WO, A Smith, **CT Wittwer**, BW Wyse, RG Hansen. Determination of plasma pantothenic acid by indirect enzyme linked immunosorbent assay. *Nutr. Res.* 10:439-448, 1990.
17. Rabkin MS, CR Kjeldsberg, **CT Wittwer**, J Marty. A comparison study of two methods of peanut agglutinin staining with S-100 immunostaining in twenty-nine cases of histiocytosis X (Langerhans' cell histiocytosis). *Arch. Path. Lab. Med.* 114:511-515, 1990.
18. Holden JA, DH Rolfsen, **CT Wittwer**. Human DNA topoisomerase II: evaluation of enzyme activity in normal and neoplastic tissues. *Biochemistry* 29:2127-2134, 1990.
19. **Wittwer CT**, GC Fillmore, DJ Garling. Minimizing the time required for DNA amplification by efficient heat transfer to small samples. *Anal. Biochem.* 186:328-331, 1990.
20. **Wittwer CT**, S Beck, M Peterson, R Davidson, DE Wilson, RG Hansen. Mild pantothenate deficiency in rats elevates serum triglycerides and free fatty acids. *J. Nutr.*, 120:719-725, 1990.
21. Rabkin MS, **CT Wittwer**, VR Soong. Flow cytometric DNA content analysis of a case of pilomatrix carcinoma showing multiple recurrences and invasion of the cranial vault. *J. Am. Acad. Derm.*, 23:104-108, 1990.
22. Hammond EH, **CT Wittwer**, J. Greenwood, WA Knape, RL Yowell, RL Menlove, C Craven, DG Renlund, MR Bristow, CW DeWitt, JB O'Connell. Relationship of OKT3 sensitization and vascular rejection in cardiac transplant patients receiving OKT3 rejection prophylaxis. *Transplantation* 50:776-782, 1990.
23. Benachenhaw D, M Cader, H Szu, L Medsker, **C Wittwer**, D Garling. AIDS viral DNA amplification by polymerase chain reaction employing primers selected by AI expert system and an ART neural network. *Proceedings of the 3rd Annual IEEE Symposium on Computer-Based Medical Systems* 3:504-511, 1990.
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26. O'Connell, JB, DG Renlund, EH Hammond, **CT Wittwer**, RL Yowell, CW DeWitt, KW Jones, WA Gay, RL Menlove and MR Bristow. Sensitization to OKT3 monoclonal antibody in heart transplantation: correlation with early allograft loss. *J. Heart Lung Transplant.*, 10:217-221, 1991.
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28. Kershnik, MM, AS Knisely, C-CJ Sun, JM Andrews, **CT Wittwer**. Cytomegalovirus infection, fetal liver disease, and neonatal hemochromatosis. *Human Pathology*, 23: 1075-80, 1992.
29. Segal, GH, **CT Wittwer**, AJ Fishleder, MH Stoler, RA Tubbs, CR Kjeldsberg. Identification of monoclonal B cell populations by rapid cycle PCR: a practical screening method for the detection of immunoglobulin gene rearrangements. *Amer. J. Path.*, 141:1291-7, 1992.
30. Weis, JH, SS Tan, BK Martin, **CT Wittwer**. Detection of rare mRNA species via quantitative RT-PCR. *Trends in Genetics*, 8:263-4, 1992.
31. Holden, JA, DH Rolfson, **CT Wittwer**. The distribution of immunoreactive topoisomerase II protein in human tissues and neoplasms. *Oncology Research*, 4:157-66, 1992.
32. **Wittwer, CT**, BC Marshall, GB Reed, JL Cherry. Rapid cycle allele-specific amplification: studies with the cystic fibrosis  $\Delta F508$  locus. *Clinical Chemistry*, 39:804-809, 1993.
33. Albro, J, KD Bauer, CL Hitchcock, **CT Wittwer**. Improved DNA content histograms from formalin-fixed paraffin-embedded liver tissue by proteinase K digestion. *Cytometry*, 14:673-678, 1993.
34. Hammond, E, R Yowell, J Greenwood, L Hartung, D Renlund, **CT Wittwer**. Prevention of adverse clinical outcome by monitoring of cardiac transplant patients for murine monoclonal CD3 antibody (OKT3). *Transplantation*, 55:1061-1063, 1993.
35. Lee HS and **CT Wittwer**. Detection of hepatitis B virus (HBV) by polymerase chain reaction (PCR). *Korean J. Clin. Path.* 13:617-623, 1993.
36. Molot RJ, TC Meeker, **CT Wittwer**, SL Perkins, GH Segal, AS Masih, RC Braylan, CR Kjeldsberg. Antigen expression and PCR amplification of mantle cell lymphomas. *Blood*, 83:1626-1631, 1994.
37. Adleberg JM and **C Wittwer**. Use of the polymerase chain reaction in the diagnosis of ocular disease. *Curr. Opin. Ophthalmol.* 6:80-85, 1995.
38. Lingenfelter B, T Fuller, L Hartung, **CT Wittwer**. HLA-B27 typing by flow cytometry. *Cytometry (Clin. Appl. Cytometry)*, 22:146-149, 1995.
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40. Mouritsen CL, **CT Wittwer**, CM Litwin, L Yang, JJ Weis, TB Martins, TD Jaskowski, HR Hill. Polymerase chain reaction detection of lyme disease: correlation with clinical manifestations and serologic responses. *Am. J. Clin. Path.*, 105:647-654, 1996.
41. Florell SR, JJ Townsend, EC Klatt, TJ Pysher, CM Coffin, **CT Wittwer**, DH Viskochil. Aprosencephaly and cerebellar dysgenesis in sibs. *Am. J. Med. Genet.* 63:542-548, 1996.
42. Endo M, PG Beatty, TM Vreeke, **CT Wittwer**, SP Singh, Parker CJ. Syngeneic bone marrow transplantation without conditioning in a patient with paroxysmal nocturnal hemoglobinuria: in vivo evidence that the abnormal stem cells have a survival advantage. *Blood*, 88:742-750, 1996.

43. Segal GH, CE Hussey, and CT Wittwer. PCR for T-cell rearrangements. *Diag. Mol. Path.*, 5:297-298, 1996.
44. Wittwer CT, MG Herrmann, AA Moss, RP Rasmussen. Continuous fluorescence monitoring of rapid cycle DNA amplification. *BioTechniques*, 22:130-138, 1997.
45. Wittwer CT, KM Ririe, RV Andrew, DA David, RA Gundry, UJ Balis. The LightCycler™: a microvolume, multisample fluorimeter with rapid temperature control. *BioTechniques*, 22:176-181, 1997.
46. Ririe KM, RP Rasmussen, and CT Wittwer. Product differentiation by analysis DNA melting curves during the polymerase chain reaction. *Anal. Biochem*, 245:154-160, 1997.
47. Swerdlow H, BJ Jones, and CT Wittwer. Fully automated DNA reaction and analysis in a fluidic capillary instrument. *Anal. Chem.*, 69:848-855, 1997.
48. Mouritsen CL, CT Wittwer, G Reed, TM Khan, TB Martins, TD Jaskowski, CM Litwin, and HR Hill. Detection of Epstein-Barr viral DNA in serum using rapid-cycle PCR. *Biochem. Mol. Med.*, 60:161-168, 1997.
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50. Bernard PS, MJ Lay, CT Wittwer. Integrated amplification and detection of the C677T point mutation in the methylenetetrahydrofolate reductase gene by fluorescence resonance energy transfer and probe melting curves. *Anal. Biochem.*, 255:101-107, 1998.
51. Morrison TB, JJ Weis, and CT Wittwer. Quantification of low-copy transcripts by continuous SYBR Green I monitoring during amplification. *BioTechniques*, 954-962, 1998.
52. Bernard PS, RS Ajioka, JP Kushner, and CT Wittwer. Homogeneous multiplex genotyping of hemochromatosis mutations with fluorescent hybridization probes. *Am. J. Pathol.*, 153:1055-1061, 1998.
53. Pritham GH and CT Wittwer. Continuous fluorescent monitoring of PCR. *J. Clin. Lig. Assay*, 21(4):404-412, 1998.
54. Lyon E, A Millson, T Phan, and CT Wittwer. Detection and identification of base alterations within the region of factor V Leiden by fluorescent melting curves. *Mol. Diag.*, 3:203-210, 1998.
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